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EXOGENOUS SPERMINE MEDIATED RESPONSES OF CATALASE AND PEROXIDASE UNDER SALT STRESS IN WHEAT (*Triticum aestivum* em Thell.)

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Abstract–Soil salinity is a major problem in many countries that adversely affects crop growth. Salinity-affected areas are not fit for crop cultivation and they only support a few halophytes. Several million ha of land were turned into saline soils due to human interference. Salinity stress is considered as one of the major environmental stresses and mitigation of it is necessary for sustainable crop production. In the present investigation four wheat cultivars differ in their tolerance to salinity *viz.* DBW 88, HD 3086, Kharchia 65 and KRL 210 were chosen with an aim to study the effect of salinity stress and subsequent spermine treatment on catalase and peroxidase enzymes which are responsible for the detoxification of reactive oxygen species (ROS). It is well documented that plant polyamine (PA), spermine is induced under stress conditions and imparts tolerance against salinity stress. Effect of exogenous spermine treatment on catalase and peroxide activities were also studied. Catalase activity increased in all four varieties and more increase was observed in Kharchia 65 and lesser increase was observed in DBW88 at 21 days after sowing (DAS). Highest catalase activity with spermine 0.5 mM concentration was observed and it was higher at 15 days after treatment (DAT). Mean catalase activity was found higher at 0 DAT in Kharchia 65 and KRL 210 and the activity was highest at 5 DAT in DBW88 and HD 3086 at 90 DAS. Peroxidase activity increased with increasing levels of salinity, but the activity was decreased with spermine treatment at 21 DAS. Highest peroxidase activity recorded in Kharchia 65 at 15 DAT and lowest was in DBW 88 at 5 DAT. Peroxidase activity decreased with spermine treatment in control and 8 dsm^{-1} and increased at 12 dsm^{-1} in all four varieties at 90 DAS.

INTRODUCTION

Global climate change-induced fluctuations in weather patterns led to uncertainty of future sustainable agriculture (Aswani *et al.,* 2020). Drought and salinity are the two important and commonly co-occurring abiotic stresses affecting plant growth and productivity worldwide (Mengesha *et al.,* 2021). Salinity in soil or irrigation water adversely affects crop growth that requires various mitigation strategies to avoid yield loss in susceptible varieties (Ehab *et al.,* 2021). However, plants have developed various strategies for overcoming the salinity stress, these include morphological, physiological and biochemical strategies. Several mechanisms work in a coordinated manner to minimize the damage due to salinity (Fariduddin *et al.,* 2013). An important possible consequence of saline stress in plants is that it causes an imbalance of cellular ions resulting in ion toxicity, nutrient imbalance, osmotic stress, alters endogenous levels of hormones and alters general metabolic processes causing excessive generation of reactive oxygen species (ROS) such as, superoxide ion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$) by a number of metabolic pathways

(Kamiab *et al.,* 2014). To counteract the toxicity of ROS, a highly efficient antioxidative defense system including both enzymatic and non-enzymatic constituents are present in plants. The formation of ROS is prevented by an antioxidant system: low molecular mass antioxidants (ascorbic acid, glutathione, carotenoids, tocopherols), ROS interacting enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), Glutathione peroxidase (GPx), catalase (CAT) and peroxidase (POX). In addition, peroxidation of lipids, a common indicator of oxidative stress also disrupts the membrane integrity of a plant cell (Farkhondeh *et al.,* 2012). The present study aims to mitigate the various effects of salinity stress through exogenous application of spermine and to study its protective action on crop plants at different days after sowing in four wheat varieties

MATERIALS AND METHODS

Plant material

Seeds of the four varieties *viz.* DBW 88, HD 3086, Kharchia 65 and KRL 210 differing in tolerance to salinity were obtained from Wheat and Barley Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar. Kharchia 65 and KRL 210 are tolerant varieties and other varieties are to be identified for tolerance to salinity.

Raising of the crop

Seeds of wheat varieties were surface sterilized by soaking them for 5 min with 0.2 per cent (*w*/*v*) solution of mercuric chloride. These surface sterilized seeds were grown in earthen pots lined with polyethylene bags filled with 6 kg dune sand in a screen house under naturally lit conditions. The nutrient solution was used to irrigate the soil supplemented with nutrients in the form of N, P and K in the ratio of 10:3:3.

Artificial saline treatment

Wheat plants were grown at two levels of salinity (8 and 12 dSm^{-1}) and control. Artificial saline waters of 8 and 12 dSm^{-1} levels were prepared for irrigation of pots by maintaining Na: Ca+Mg ratio as 1:1; Ca: Mg as 1:3 and that of Cl: SO_4 as 7:3. The respective cations and anions ratio and salt levels were maintained by dissolving required quantities of chloride & sulfate salts of calcium, magnesium, and sodium in distilled water. Plants were irrigated with distilled water to maintain the salinity levels.

Spermine treatment

Spermine at 0.5 and 1.0 mM concentration was sprayed over the plants at 21 and 90 days after sowing.

Antioxidant enzymes

Extraction

Two gram of leaf sample was homogenized using pre-chilled pestle and mortar in 5 ml of cold extraction buffer containing 0.1 M phosphate buffer (pH 7.0), 2.5 mM DDT and 1 mM EDTA. Then, the homogenate was centrifuged at 10,000 rpm for 30 min. The whole procedure of preparation of enzyme extract was carried out at 0 - 4 °C. The supernatant was used for enzymatic assay for determining the activity of catalase (CAT), peroxidase (POX).

Catalase (EC 1.11.1.6)

Catalase activity was measured by the method of Sinha (1972). To 0.55 ml of 0.1 M potassium phosphate buffer (pH 7.0) 0.4 ml of 0.2 M H_2O_2 and 50 µl of enzyme extract was added. The assay mixture without enzyme extract acted as a control. The reaction mixture was mixed thoroughly and incubated at 37° C for one min. and terminated by adding 3 ml mixture of 5 % (w/v) potassium dichromate and glacial acetic acid (1:3 v/v). Then, the tubes were kept in boiling water bath for 10 min. cooled and absorbance was recorded at 570 nm using dichromate: acetate solution as blank. The amount of H_2O_2 in the reaction mixture was determined by subtracting the absorbance of test samples from that of control. One unit of enzyme activity was defined as the amount of enzyme which catalyzed the oxidation of 1 µ mole H_2O_2 per min.

Peroxidase (EC 1.11.1.7)

The peroxidase enzyme assay was done according to the method of Shannon *et al.* (1966). The reaction mixture contained 2.75 ml of 50 mM phosphate buffer (pH 6.5), 0.1 ml of 0.5 % hydrogen peroxide, and 0.1 ml of 0.2 % O-dianisidine and 0.05 ml of enzyme extract. The reaction was initiated by the addition of 0.1 ml of H_2O_2. The assay mixture without H_2O_2 served as blank. Change in absorbance was followed at 430 nm for 3 min. One unit of POX was defined as the amount of enzyme required to cause O.D. change per minute.

RESULTS

Catalase

Salt- stress induced activity of catalase in all the

varieties and the level of increase was more pronounced in Kharchia 65 (from 4.54 units g^{-1} FW in control to 5.79 and 8.33 units g^{-1} FW in 8 dSm^{-1} and 12 dSm^{-1} respectively) and lesser increase was observed in DBW 88 (from 4.01 units g^{-1} FW in control to 4.81 and 5.69 units g^{-1} FW at 8 dSm^{-1} and 12 dSm^{-1} respectively) at 21 DAS (Table 1). The highest mean activity of CAT was obtained with Spm treatment of 0.5 mM concentration (6.31, 8.24, 10.89 units g^{-1} FW in control, 8 dSm^{-1} and 12 dSm^{-1} respectively), it was higher at 15 DAT in all the varieties at 21 DAS. Results presented in Table 2 demonstrates that the salt stress induced an increase in the activity of catalase in flag leaf of wheat at 90 DAS. There was an enhancement in the mean CAT activity with Spm treatment in control (10.43, 11.78, and 11.49 units g^{-1} FW in Spm^{0}, $Spm^{0.5mM}$ and $Spm^{1.0mM}$ respectively) and in salt-stressed wheat leaves (13.24, 14.22, 14.20 units g^{-1} FW inSpm^{0}, $Spm^{0.5mM}$ & $Spm^{1.0mM}$ at 8 $dSm^{-1}$and 17.34, 19.97, 19.55 units g^{-1} FW in Spm^{0}, $Spm^{0.5mM}$& $Spm^{1.0mM}$ at 12 dSm^{-1} respectively) at 90 DAS. The mean CAT was found higher at 0 DAT in Kharchia 65 & KRL 210 and activity was higher at 5 DAT in DBW 88 & HD 3086 at 90 DAS. There was a larger increase in the catalase activity with increasing levels of salinity at both 21 DAS and 90 DAS in all the varieties but the increase was observed more in the case of Kharchia 65 followed by KRL 210, HD 3086 and DBW 88 (Table 1&2). The saline and Spm treatment at 8 dSm^{-1} + 0.5 mM in DBW 88 and HD 3086 initially decreased the CAT activity (from 0 DAT to 10 DAT) whereas the same treatment showed an increase in CAT activity in Kharchia 65 and KRL 210 varieties. Spermine application of 0.5 mM concentration was found to be more effective in increasing the activity of CAT in control and under saltstress in all the varieties at both 21 DAS and 90 DAS.

Peroxidase

As evident from the data in Table 3 that the peroxidase activity was enhanced with increasing

Table 1. Changes in catalase activity in wheat flag leaf under different levels of salinity and spermine treatment at 21 days after sowing

Catalase activity (units g^{-1} FW)											
Variety	21 DAS	Saline and spermine treatment									Mean
		Control			8 dSm^{-1}			12 dSm^{-1}			
		Spm^{0}	Spm 0.5mM	Spm 1.0mM	Spm^{0}	Spm 0.5mM	Spm 1.0mM	Spm^{0}	Spm 0.5mM	Spm 1.0mM	
DBW 88	0 DAT	4.01	4.01	4.01	4.81	4.81	4.81	5.69	5.69	5.69	4.84
	5 DAT	4.71	5.26	4.93	6.04	5.90	6.31	6.46	7.47	7.97	6.12
	10 DAT	5.20	5.70	5.38	6.87	6.65	6.45	7.20	8.18	8.18	6.65
	15 DAT	8.47	9.13	8.73	10.85	10.70	9.91	11.45	12.73	12.31	10.48
HD 3086	0 DAT	4.33	4.33	4.33	5.12	5.12	5.12	6.37	6.37	6.37	5.27
	5 DAT	5.12	5.68	5.37	6.43	6.29	6.68	7.27	8.37	8.91	6.68
	10 DAT	5.47	5.96	5.66	7.04	6.83	6.65	7.83	8.85	8.86	7.02
	15 DAT	9.09	9.77	9.40	11.38	11.24	10.51	12.73	14.09	13.65	11.32
Kharchia 65	0 DAT	4.54	4.54	4.54	5.79	5.79	5.79	8.33	8.33	8.33	6.22
	5 DAT	5.39	5.90	5.63	6.49	8.22	8.53	9.84	11.91	12.68	8.29
	10 DAT	6.08	6.56	6.29	7.62	9.94	8.51	11.69	14.50	14.00	9.47
	15 DAT	10.17	10.84	10.49	12.58	18.13	14.88	19.14	24.31	23.11	15.96
KRL 210	0 DAT	3.72	3.72	3.72	4.54	4.54	4.54	6.21	6.21	6.21	4.82
	5 DAT	4.51	4.98	4.73	5.25	6.40	6.61	7.49	8.87	9.39	6.47
	10 DAT	5.06	5.50	5.26	6.09	7.64	6.69	8.81	10.68	10.34	7.34
	15 DAT	8.39	9.00	8.69	9.99	13.65	11.50	14.32	17.73	16.94	12.25
Mea n		5.89	6.31	6.07	7.31	8.24	7.72	9.43	10.89	10.81	

CD at 5 % level								Where,	
a →	0.16	ab →	0.28	bd →	0.28	acd →	0.56	a →	Varieties
b →	0.14	ac →	0.32	cd →	0.28	bcd →	0.48	b →	Artificial saline treatment
c →	0.14	ad →	0.32	abd→	0.56	abcd →	NS	c →	Spermine treatment
d →	0.16	bc →	0.24	abc →	0.48			d →	Days after treatment (DAT)

levels of salinity but the activity was decreased with Spm treatment at 21 DAS. The POX activity increased from 41.53 units g^{-1} FW in control to 51.17 units g^{-1} FW in 8 dSm^{-1} and 72.71 units g^{-1} FW in 12 dSm^{-1} at 21 DAS. The application of Spm decreased the mean activity of peroxidase in control (from 41.53 units g^{-1} FW to 39.71 and 39.50 units g^{-1} FW in $Spm^{0.5mM}$ and $Spm^{1.0mM}$ respectively) and in salt-stressed wheat leaves (from 51.17 to 47.23 and 49.24 units g^{-1} FW in $Spm^{0.5mM}$ and $Spm^{1.0mM}$ at 8 dSm^{-1} and from 72.71 to 59.16 and 61.05 units g^{-1} FW in $Spm^{0.5mM}$ and $Spm^{1.0mM}$ at 12 dSm^{-1} respectively). The highest POX activity was recorded in Kharchia 65 (94.27 units g^{-1} FW in 12 dSm^{-1}) at 15 DAT whereas activity was lowest in DBW 88 (33.00 units g^{-1} FW in control + $Spm^{1.0mM}$) at 5 DAT. The mean activity of POX was highly pronounced at 15 DAT (62.14, 57.92, 52.13 and 50.37 units g^{-1} FW in Kharchia 65, KRL 210,DBW 88 and HD 3086 respectively). In contrary to the observations at 21 DAS (Table 3) the POX activity decreased with the Spm treatment in control and 8 dSm^{-1} and increased at higher levels of salinity (12 dSm^{-1}) in all the varieties at 90 DAS (Table 4). However, the mean POX activity increased with the increasing levels of salinity from 51.32 units g^{-1} FW in control to 65.64 and 95.13 units g^{-1} FW at 8 dSm^{-1} and 12 dSm^{-1} respectively. The maximum activity of POX was recorded in Kharchia 65 (146.30 units g^{-1} FW) in 12 dSm^{-1} + $Spm^{1.0mM}$and minimum activity was found in HD 3086 (37.84 units g^{-1} FW) in control + $Spm^{0.5mM}$ at 10 DAT.

DISCUSSION

The first line enzymatic defense against ROS, SOD converts O_2– to H_2O_2 and H_2O_2 is readily detoxified by catalase (CAT), a heme-containing enzyme, through conversion of H_2O_2 to H_2O and O_2. Under salinity, an increase in CAT activity was observed in leaves of all the varieties but the increase was observed more in the case of Kharchia 65 and KRL 210 (Table 1 and 2). Nouri *et al.* (2015) also reported

Table 2. Changes in catalase activity in wheat flag leaf under different levels of salinity and spermine treatment at 90 days after sowing

Catalase activity (units g^{-1} FW)											
Variety	90 DAS	Saline and spermine treatment									Mean
		Control			8 dSm^{-1}			12 dSm^{-1}			
		Spm^0	Spm 0.5mM	Spm 1.0mM	Spm^0	Spm 0.5mM	Spm 1.0mM	Spm^0	Spm 0.5mM	Spm 1.0 mM	
DBW 88	0 DAT	9.20	10.28	11.46	13.62	11.44	11.98	13.86	15.19	15.66	12.52
	5 DAT	10.06	11.80	10.87	14.70	13.12	12.51	14.66	16.93	16.33	13.44
	10 DAT	7.75	8.87	8.16	11.07	10.57	9.17	11.10	13.27	11.77	10.19
	15 DAT	7.23	8.37	7.65	9.77	10.29	8.12	10.14	12.14	10.38	9.34
HD 3086	0 DAT	10.31	11.44	12.59	14.72	12.57	13.11	16.02	17.51	18.02	14.03
	5 DAT	10.70	12.39	11.52	15.08	13.61	13.04	16.09	18.48	17.85	14.31
	10 DAT	8.90	10.07	9.37	12.29	11.78	10.37	13.17	15.63	13.93	11.72
	15 DAT	8.17	9.34	8.64	10.73	11.24	9.09	11.85	14.08	12.11	10.58
Kharchia 65	0 DAT	14.09	15.42	16.70	17.04	19.49	22.19	25.96	28.07	34.00	21.44
	5 DAT	12.85	14.57	13.72	14.94	18.88	20.61	24.84	29.59	28.83	19.87
	10 DAT	13.36	14.86	14.00	15.64	19.41	20.08	25.40	30.10	26.45	19.92
	15 DAT	10.94	12.28	11.51	12.78	16.29	15.35	20.38	24.14	22.87	16.28
KRL 210	0 DAT	11.79	13.01	14.19	13.77	15.41	17.21	19.74	21.16	25.12	16.82
	5 DAT	11.06	12.68	11.88	12.50	15.21	16.40	19.31	22.59	22.07	15.97
	10 DAT	10.66	11.98	11.22	12.11	14.52	14.95	18.34	21.35	19.02	14.91
	15 DAT	9.82	11.15	10.38	11.14	13.66	12.98	16.60	19.30	18.39	13.71
Mea n		10.43	11.78	11.49	13.24	14.22	14.20	17.34	19.97	19.55	

CD at 5 % level								Where,	
a →	0.28	ab →	0.48	bd →	0.48	acd →	NS	a →	Varieties
b →	0.24	ac →	0.48	cd →	0.48	bcd →	0.83	b →	Artificial saline treatment
c →	0.24	ad →	0.56	abd →	NS	abcd →	NS	c →	Spermine treatment
d →	0.28	bc →	0.42	abc →	0.83			d →	Days after treatment (DAT)

Table 3. Changes in peroxidase activity in wheat flag leaf under different levels of salinity and spermine treatment at 21 days after sowing

Peroxidase activity (units g^{-1} FW)											
Variety	21 DAS	Saline and spermine treatment									Mean
		Control			8 dSm^{-1}			12 dSm^{-1}			
		Spm^0	Spm 0.5mM	Spm 1.0mM	Spm^0	Spm 0.5mM	Spm 1.0mM	Spm^0	Spm 0.5mM	Spm 1.0 mM	
DBW 88	0 DAT	35.58	35.58	35.58	42.33	42.33	42.33	58.66	58.66	58.66	45.52
	5 DAT	35.07	33.25	33.00	42.82	40.10	39.68	58.25	49.84	48.43	42.27
	10 DAT	43.11	40.53	41.17	53.10	47.84	50.08	72.49	55.25	57.85	51.27
	15 DAT	44.53	42.08	42.66	54.31	48.15	53.07	73.63	52.78	58.00	52.13
HD 3086	0 DAT	34.45	34.45	34.45	40.85	40.85	40.85	54.57	54.57	54.57	43.29
	5 DAT	33.72	32.11	31.93	40.88	38.36	37.98	55.63	47.71	46.38	40.52
	10 DAT	41.62	39.33	39.94	50.88	46.01	48.08	69.50	53.19	55.65	49.36
	15 DAT	43.13	40.95	41.50	52.23	46.50	51.08	70.85	51.05	56.01	50.37
Kharchia 65	0 DAT	41.51	41.51	41.51	50.17	50.17	50.17	76.54	76.54	76.54	56.07
	5 DAT	40.39	37.96	36.11	51.12	47.52	47.09	75.11	63.37	61.17	51.10
	10 DAT	49.41	45.98	45.41	63.15	56.08	59.48	93.18	68.20	72.22	61.46
	15 DAT	50.96	47.71	47.11	64.40	56.06	63.28	94.27	63.72	71.79	62.14
KRL 210	0 DAT	38.98	38.98	38.98	46.70	46.70	46.70	70.22	70.22	70.22	51.97
	5 DAT	37.88	36.03	34.56	47.44	44.30	43.85	68.81	58.78	56.40	47.56
	10 DAT	46.27	43.64	43.19	58.48	52.26	55.22	85.20	63.33	66.56	57.13
	15 DAT	47.82	45.34	44.86	59.79	52.41	58.80	86.42	59.43	66.38	57.92
Mea n		41.53	39.71	39.50	51.17	47.23	49.24	72.71	59.16	61.05	

CD at 5 % level								Where,	
a →	0.37	ab →	0.65	bd →	0.65	acd →	NS	a →	Varieties
b →	0.32	ac →	0.65	cd →	0.65	bcd →	1.12	b →	Artificial saline treatment
c →	0.32	ad →	0.74	abd →	1.29	abcd →	NS	c →	Spermine treatment
d →	0.37	bc →	0.56	abc →	1.12			d →	Days after treatment (DAT)

higher activities of CAT under salinity in wheat genotypes. The present results are in accordance with observations of various workers (Mandhania *et al.* 2006; Bhutta, 2011; Amjad *et al.* 2014) studied in wheat. Spermine is able to counteract damage from abiotic stresses and has been shown to protect plants from variety of environmental insults (Mahadi *et al.*, 2021). In the present study exogenous spermine treatment further enhanced the CAT activity at the higher level of salinity (12 dSm^{-1}) in all the varieties. However, CAT activity was initially decreased by Spm treatment of 0.5 mM in susceptible varieties at a lower level of salt stress (8 dSm^{-1}). Kamiab *et al.* (2014) studied the exogenous application of free PA in pistachio seedlings under salt stress and reported that the activity of SOD and CAT increased with salinity and application of PAs significantly increased the activity of these enzymes. Saeidnejad *et al.* (2016) studied the effect of Spm in wheat seedlings under salinity and showed that PAs further increased the activity of CAT during salt stress which was more pronounced in tolerant variety. Abdelaleim *et al.*, 2018 had reported that exogenous spermine regulate the activity of catalase and efficiently modulate transcription levels in wheat seedlings under salt stress.

Peroxidases (POX) are a group of enzymes play key roles in scavenging excess hydrogen peroxide (Peisen *et al.*, 2020). In the present study, the POX activity increased with increasing salinity levels in all the varieties at 21 DAS and 90 DAS (Table 3 & 4). However, the increment with salinity was more in Kharchia 65 and KRL 210 when compared with susceptible varieties. Sheokand *et al.* (2008) also reported an increase in activities of CAT and POX with salinity stress in chickpea. A substantial increase in the activities of antioxidant enzymes such as SOD, POX and CAT in salt tolerant variety under salinity stress in wheat has been reported by Ashraf *et al.* (2012). However, the activity of POX varied at different levels of salinity and stage of Spm application. Irrespective of salinity stress POX

Table 4. Changes in peroxidase activity in wheat flag leaf under different levels of salinity and spermine treatment at 90 days after sowing

Peroxidase activity (units g^{-1} FW)											
Variety	90 DAS	Saline and spermine treatment									Mean
		Control			8 dSm^{-1}			12 dSm^{-1}			
		Spm^0	Spm 0.5mM	Spm 1.0mM	Spm^0	Spm 0.5mM	Spm 1.0mM	Spm^0	Spm 0.5mM	Spm 1.0 mM	
DBW 88	0 DAT	50.76	48.73	47.72	62.78	59.25	58.52	90.60	94.48	100.59	68.16
	5 DAT	50.00	46.50	47.40	63.58	56.19	58.59	87.30	99.59	97.60	67.42
	10 DAT	43.21	39.76	41.27	52.78	46.41	51.09	73.55	84.71	80.82	57.07
	15 DAT	44.34	41.20	42.97	55.69	46.68	53.28	73.21	82.06	78.53	57.55
HD 3086	0 DAT	48.83	46.64	45.54	61.83	58.01	57.22	91.90	96.09	102.70	67.64
	5 DAT	47.97	44.20	45.17	62.61	54.64	57.23	88.18	101.44	99.29	66.75
	10 DAT	41.57	37.84	39.47	51.92	45.03	50.09	74.36	86.43	82.22	56.55
	15 DAT	42.82	39.41	41.33	55.14	45.36	52.52	74.15	83.75	79.92	57.16
Kharchia 65	0 DAT	63.73	60.59	59.03	82.29	77.37	76.20	129.75	137.82	146.30	92.56
	5 DAT	59.49	54.36	55.68	79.36	68.90	72.57	118.05	139.13	134.23	86.86
	10 DAT	56.55	50.99	53.42	71.96	61.92	69.76	108.97	129.89	121.54	80.56
	15 DAT	53.72	49.04	51.68	70.64	57.34	67.58	99.89	115.42	108.40	74.86
KRL 210	0 DAT	59.45	57.10	55.86	75.55	71.53	70.27	116.73	125.11	131.08	84.74
	5 DAT	55.63	51.70	52.74	72.92	64.00	67.01	106.58	126.40	120.64	79.74
	10 DAT	52.95	48.65	50.59	66.37	57.73	64.45	98.60	118.19	109.54	74.12
	15 DAT	50.16	46.56	48.65	64.85	53.37	62.19	90.24	104.87	97.63	68.72
Mea n		51.32	47.70	48.66	65.64	57.73	61.79	95.13	107.84	105.69	

CD at 5 % level								Where,	
a →	0.49	ab →	0.86	bd →	0.86	acd →	NS	a →	Varieties
b →	0.43	ac →	NS	cd →	0.86	bcd →	1.48	b →	Artificial saline treatment
c →	0.43	ad →	0.99	abd →	1.71	abcd →	NS	c →	Spermine treatment
d →	0.49	bc →	0.74	abc →	1.48			d →	Days after treatment (DAT)

activity decreased in leaves at 21 and 90 DAS and an increase in activity of POX was observed at higher levels of salinity with Spm treatment. The results obtained in present investigation are consistent with the results of Tanou *et al.* (2014) who showed that Spm to NaCl-treated plants increased the activity of CAT, MDHAR, DHAR whereas Spm application decreased the POX and APX activities in citrus plants. In contrary, Li *et al.* (2015) showed that Spd application to salinized nutrient solution increased the antioxidant enzyme activities, including SOD, POX APX and GR in cucumber seedlings. Yassin *et al.,* (2019) had also showed an increased peroxidase activity in the leaf of genotype 'Misr2' under salt stress. The results are also in agreement with the results of Muhammead *et al.,* (2020) who reported significant cultivar dependent changes in the activities of catalase and peroxidase in roots and leaves after salinity treatment in wheat and barley.

CONCLUSION

- The enhancement in the activities of POX and CAT were more in tolerant varieties than susceptible varieties with increasing levels of salinity.
- Exogenous Spm treatment triggered the activities of POX and CAT and varied with tolerance to different levels of salinity and stage of Spm application. Thus, Spm efficiently protected the plant tissue from damaging effects of O_2 and H_2O_2 induced by high levels of salt stress.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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